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Trends in Immunotherapy of Fungal Infections

B. J. Kullberg

Fungal infections are the primary cause of mortality in patients with severely impaired host defense mechanisms, such as neutropenic patients with acute leukemia or those who have undergone bone marrow transplantation. In view of the unacceptably high mortality due to disseminated candidiasis, it is rational to focus on augmentation of host defense mechanisms in addition to conventional antifungal therapy. In vitro, a variety of immunomodulators, including tumor necrosis factor, interferon-g, and the hematopoietic growth factors, enhance the killing of *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. Various studies have demonstrated beneficial effects of immunomodulatory therapy in animal models of disseminated candidiasis. For further preclinical and clinical studies, recombinant interferon-g, interleukin-1, granulocyte colony-stimulating factor, and the other hematopoietic growth factors are currently the most promising immunomodulators.

Recently, a variety of agents have been investigated as potential immunomodulators in the treatment of sepsis syndrome. They include: antibodies to bacterial lipopolysaccharide (LPS) and to cytokines, recombinant cytokines as well as cytokine antagonists, monoclonal antibodies to the integrin family, and modulators of the coagulation cascade. Although it may be tempting to apply a similar concept to the immunomodulation of disseminated fungal infections, e.g., candidemia, the important pathophysiological differences between disseminated bacterial infection and disseminated fungal infection must be taken into consideration. Experimental gram-negative infection in animal models leads to high concentrations of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1ß (IL-1ß). Specific inhibitors of either TNF-α or interleukin-1 (IL-1) effectively prevent mortality in these models, underscoring the important role of pro-inflammatory cytokines in the pathogenesis of lethal sepsis (1).

In a similar model of lethal infection of experimental animals with *Candida albicans*, only very low and gradually increasing concentrations of pro-inflammatory cytokines were found, and treatment with cytokine inhibitors or antagonists did not influence the outcome of candidemia, indicating that these animals did not die from cytokinemia (2, 3). Apparently, the concept of lethal cytokinemia, which is pivotal in the design of trials for sepsis syndrome, does not apply to candidemia. Although candidemia presenting as fulminant sepsis has been described in a minority of patients, no data exist on cytokine patterns in this group. It may be that other factors such as underlying disease contribute to the activation of the cytokine network in this specific subgroup, leading to further production and release of pro-inflammatory cytokines upon subsequent challenge with *Candida albicans*.

In contrast, the administration of monoclonal antibodies to TNF-α leads to increased mortality in models of disseminated candidiasis and to increased outgrowth of *Candida albicans* compared to that seen in controls (2, 4). The effect of anti-TNF-α is not seen in neutropenic animals, which supports observations indicating that in vitro TNF-α is required to activate polymorphonuclear leukocytes (PMNs) for killing of *Candida* spp. and that its inhibition restricts the intracellular killing of the yeasts. Therefore, it will prove more productive to direct immunotherapy toward enhancing host defense mechanisms by increasing the numbers of phagocytic cells, modulating the kinetics of these cells at the site of in-

Catholic University Nijmegen, Department of Medicine (541), University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Table 1: Antifungal effects of selected cytokines and hematoppoietic growth factors in vitro and in animal models.

Cytokine/ growth factor	*Candida* spp.		*Aspergillus* spp.		*Cryptococcus* spp.	
	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo
TNF-α	+	+	NA	NA	+	+
IFN-γ	+	+	+	+	+	+
IL-1	no effect	+	NA	NA	NA	NA
G-CSF	+	+	+	+	+	+
GM-CSF	+	+	+	NA	+	NA
M-CSF	+	+	NA	NA	+	NA

G-CSF, granulocye colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon-γ; IL-1, interleukin 1; M-CSF, macrophage colony-stimulating factor; NA, no data availabe; TNF-α, tumor necrosis factor-α; +, Beneficial effect has been demonstrated on killing in vitro or on course of infection in animal model.

fection, or activating phagocytes to kill the pathogenic fungal organisms more effectively (Table 1). These effects may be partly controlled by the differentiation of T-helper cell subsets. The T_{H1}/T_{H2} balance itself may be the target of immunotherapy as well. Other potential targets might include modulating humoral fungicidal factors, the interaction between circulating fungal pathogens and endothelial cells, and factors that affect either the pharmacokinetics or the pharmacodynamics of antifungal agents. The nature and stage of the infection determine the type of effector cells that therapy should target (reviewed in 5). Alveolar macrophages provide the first line of host defense against airborne infection with *Aspergillus* conidia, and activation of these cells may be considered a preventive measure. After *Aspergillus* germinates and the hyphae invade the pulmonary tissue, PMNs become the main effector cells involved, killing the mycelia of *Aspergillus* by secreting oxidative and nonoxidative metabolites. Therefore, immunotherapy at this stage of the disease should focus on either the number or the function of PMNs.

Invasive cryptococcosis is strongly associated with disorders of lymphocyte function, such as AIDS, since T-cell specific immunity is the most important host defense mechanism against *Cryptococcus neoformans*. Depletion of natural killer cells does not affect the course of experimental cryptococcosis or the load of *Cryptococcus neoformans* in the organs except for the lungs, indicating that natural killer cells play a limited and early role in the host defense to *Cryptococcus neoformans* in the lung. However, in the early phase of the infection, before cell-mediated immunity is activated, macrophages and PMNs also play a role in host defense to these yeasts; immunotherapy could focus on these defense mechanisms as well.

In candidiasis, the clinical manifestation of the disease is crucial in establishing which host defense mechanism should be addressed. Whereas T-cell-mediated immunity is involved in candidiasis of the mucosal surfaces, PMNs play the primary role in host defense against invasive or disseminated candidiasis, as demonstrated by the various patient groups at risk for these infections. Disseminated candidiasis is relatively rare in patients with AIDS and other diseases affecting T-cell function, which suggests that cell-mediated immunity is of minor importance in the host defense against this form of infection. It is generally accepted that PMNs should be the primary object of strategies to enhance host defenses against candidemia and acute disseminated or invasive disease. In chronic disseminated candidiasis or mucosal infection, efforts at enhancing host defenses should include activation of macrophages and modulation of T-helper cell differentiation.

Pro-Inflammatory Cytokines

Interferon-γ (IFN-γ) has been shown to be an essential cytokine for activating fungicidal activities of phagocytes in vitro (6, 7). Administration of a single dose of recombinant IFN-γ to mice with disseminated candidiasis significantly reduced the outgrowth of *Candida albicans* in their organs, even when the infection had been established for several days (8). Of note, a single injection of IFN-γ resulted in a beneficial effect that persisted for at least one week. These data indicate that it might be feasible to study combination therapy of a conventional antifungal agent and recombinant IFN-γ in treating disseminated candidiasis. In severely neutropenic mice, treatment with IFN-γ did not alter the course of disseminated candidiasis, suggesting that the effect of IFN-γ is mediated through the activation of PMN (8). This is in agreement with the observation that neutrophils isolated from IFN-γ-treated mice show enhanced intracellular killing of *Candida albicans* (8). A re-

cent study showed that administering recombinant IFN-γ has a beneficial effect on the course of lethal disseminated cryptococcosis in rats (9). Although no specific clinical trials have been performed to establish the role of recombinant IFN-γ in the treatment of invasive mycoses, a beneficial effect of IFN-γ has been demonstrated in patients with chronic granulomatous disease, a rare specific disorder of phagocytes. In a large, prospective, randomized, placebo-controlled trial, the incidence of invasive aspergillosis in a group of patients with chronic granulomatous disease who received IFN-γ prophylaxis was significantly reduced from 24 to 4% in two years, and PMNs from IFN-γ-treated patients displayed enhanced killing of *Aspergillus* in vitro (10, 11). Taken together, addition of IFN-γ to the antifungal regimen appears to be effective in treating infections with *Candida*, *Cryptococcus* or *Aspergillus* spp. in non-neutropenic patients. Further prospective randomized clinical studies should be considered in these patient groups.

Recently, recombinant IL-1 has been administered to patients in order to promote the hematopoietic response after bone marrow transplantation (12, 13). Although this cytokine has considerable side effects, such as fever, chills, and hypotension, clinical studies evaluating the specific effect of IL-1 on the host defense against *Candida* infection may be warranted. In an experimental model it has been demonstrated that a single injection of either recombinant interleukin 1α (IL-1α) or interleukin 1β (IL-1β) protects neutropenic mice from lethal disseminated candidiasis (14). Treatment also significantly decreases the numbers of *Candida albicans* in the kidneys and spleen of infected normal mice and of mice rendered immunocompromised by cyclophosphamide, hydrocortisone acetate, or total body irradiation (15). A similar protective effect of IL-1 has been demonstrated in models of bacterial infection caused by a variety of species (reviewed in 16). Although the mechanism of IL-1 induced protection has not been fully elucidated, modulation of cytokine receptors and induction of acute phase proteins and other humoral factors contribute to its effect (16, 17), which is independent of the presence or activation of phagocytic cells (15).

Interleukin-12 (IL-12) is produced very early in the infective process, upon antigen presentation by macrophages, and it enhances both the production of IFN-γ and T-cell differentiation. In a recent study recombinant IL-12 was administered to rats with disseminated cryptococcosis (18). After two weeks of infection, treatment with either IL-12 or fluconazole reduced the outgrowth of organisms in the brain, and the combination of IL-12 and fluconazole had an additive effect. In the liver fluconazole failed completely to contain the infection, whereas treatment with IL-12 was effective in reducing the outgrowth of *Cryptococcus neoformans* (18).

Anti-Inflammatory Cytokines

The release of IFN-γ and the activation of macrophages are both inhibited by anti-inflammatory cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10). Failure to clear a subacute or chronic infection with *Candida albicans* corresponds with sustained production of IL-4 and IL-10 in susceptible mouse strains, whereas so-called constitutively resistant strains produce significantly less IL-4 and IL-10 (19). Neutralization of either of these cytokines by specific monoclonal antibodies augments host resistance, leads to increased survival of infected mice, and enhances the ability of their macrophages to kill *Candida albicans* in vitro (19, 20). In a different approach, recombinant soluble receptors to IL-4 (sIL-4R) have been cloned (21). The sIL-4R circulate in the bloodstream and are able to bind and neutralize circulating IL-4. Treatment of mice with recombinant sIL-4R was able to cure potentially lethal subacute disseminated infection caused by *Candida albicans* (21).

Hematopoietic Growth Factors

Colony-stimulating factor (CSF) is able to augment the numbers of circulating phagocytes and their precursors in the bone marrow (22). Therefore, granulocyte colony-stimulating factor (G-CSF) is now being administered to donors in order to elicit neutrophils that can be transfused into patients with life-threatening fungal infections (unpublished data). Moreover, CSF has been shown to enhance activation of the fungicidal capacity of phagocytic cells in vitro. Incubation of macrophages with macrophage colony-stimulating factor (M-CSF) enhances the killing of *Candida* spp. and *Cryptococcus* spp. (23, 24). Treatment of chronic disseminated candidiasis in rats with M-CSF has been shown to reduce the outgrowth of *Candida albicans* (25). Neutrophil function is enhanced by G-CSF and granulocyte macrophage

colony-stimulating factor (GM-CSF), and incubation of PMNs with either growth factor leads to increased killing of *Candida* spp. or *Aspergillus* spp. in vitro (7, 26). In an experimental model in vitro, the ability of PMNs to damage *Aspergillus* hyphae was abrogated by incubating the cells with corticosteroids, underscoring the fact that steroids are a risk factor for invasive aspergillosis (27). Incubating PMNs with G-CSF effectively reversed the deleterious effect of steroids in this model, reinstating their capacity to kill *Aspergillus* in vitro (27). Incubating PMNs with IFN-γ or the combination of G-CSF and IFN-γ had similar effects (28). A pilot study is therefore being conducted in the USA on the treatment of patients with proven aspergillosis with the combination of recombinant G-CSF and IFN-γ. Other studies have shown that PMNs isolated from AIDS patients are less able to kill bacterial pathogens as well as *Candida albicans* in vitro (28). Administering a single dose of recombinant G-CSF to AIDS patients effectively restored the capacity of their peripheral blood PMNs to kill *Candida albicans* to normal levels (29).

In animal models of disseminated candidiasis, various studies have shown a beneficial effect of recombinant G-CSF given prophylactically. Repeated administration of G-CSF effectively prevents the development of cyclophosphamide-induced neutropenia and protects animals after a lethal challenge with *Candida albicans* (30). Fewer data exist on the therapeutic administration of G-CSF after the microorganisms have disseminated and the experimental infection has become established. No effect of G-CSF was found in a carefully designed model of chronic disseminated candidiasis in persistently neutropenic rabbits (D. Arenberg et al., General Meeting of the American Society for Microbiology, Washington DC, 1990, Abstract no. F90). In contrast, in non-neutropenic animals, G-CSF was shown to be effective even when administration was begun after infection (31). Moreover, combination therapy of recombinant G-CSF with various antifungal agents appeared to be synergistic. A multicenter, double-blind, randomized phase II trial is currently being conducted in Europe to assess the safety and preliminary efficacy of G-CSF in combination with fluconazole in treating invasive candidiasis and candidemia in non-neutropenic patients.

Future Directions

In conclusion, the administration of recombinant G-CSF or the combination of G-CSF and IFN-γ in addition to conventional antifungal therapy currently comes closest to realizing the goals in the immunotherapy of invasive fungal infections. Various issues are to be addressed in the near future: (i) following the ongoing phase II trial of G-CSF in disseminated candidiasis, clinical trials may be designed to investigate the use of G-CSF or the combination of G-CSF and IFN-γ in disseminated candidiasis or invasive aspergillosis in non-neutropenic as well as neutropenic cancer patients; (ii) additional preclinical studies should address the effects of recombinant GM-CSF and M-CSF when given as a therapeutic modality in animal models of candidiasis and aspergillosis, and clinical studies may be designed to address this purpose as well; (iii) clinical studies with recombinant IFN-γ administered for prophylaxis or treatment of either candidiasis or aspergillosis in non-neutropenic subjects are warranted; (iv) a clinical trial to evaluate the ability of recombinant IL-1 to prevent fungal infections in either neutropenic or non-neutropenic patients at risk may be considered; and (v) more preclinical data are required on the effects of recombinant IL-1 and IFN-γ in invasive aspergillosis.

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